

Intravenous Lidocaine Attenuates Acute Lung Injury Induced by Hydrochloric Acid Aspiration in Rabbits

Kahoru Nishina, M.D.,* Katsuya Mikawa, M.D.,† Yumiko Takao, M.D.,‡ Makoto Shiga, M.D.,‡ Nobuhiro Maekawa, M.D.,§ Hidefumi Obara, M.D.||

Background: Neutrophils play a crucial role in the pathogenesis of acid-induced acute lung injury. Lidocaine inhibits the function of neutrophils. This study aimed to determine whether lidocaine attenuates acute lung injury induced by hydrochloric acid (HCl) instillation.

Methods: In study 1, rabbits were divided into four groups ($n = 7$ each). Lung injury was induced by intratracheal HCl (0.1 N, 3 ml/kg) in two groups. The other two groups received saline intratracheally. Lidocaine given intravenously (2 mg/kg bolus + 2 mg · kg⁻¹ · h⁻¹ infusion) was started 10 min before intratracheal instillation in one HCl and one saline group, and saline was given intravenously in the other two groups. In study 2, rabbits (four groups of seven animals each) received HCl (0.1 N, 3 ml/kg) intratracheally. Treatment with intravenous lidocaine was started 10 min before, 10 min after, or 30 min after acid instillation, or saline was given intravenously 10 min before instillation.

Results: In study 1, HCl caused deterioration of the partial pressure of oxygen (Pa_{O₂}), lung leukosequestration, decreased lung compliance, and increased the lung wet-to-dry weight ratio and albumin, interleukin-6 (IL-6), and IL-8 levels in bronchoalveolar lavage fluid. Lidocaine pretreatment attenuated these changes. Hydrochloric acid increased superoxide anion production by neutrophils and caused morphologic lung damage, both of which were lessened by lidocaine. In study 2, lidocaine given 10 min after acid instillation was as effective as pretreatment in Pa_{O₂}, lung mechanics, and histologic examination. However, Pa_{O₂} changes in lidocaine 30 min after injury were similar to those in saline given intravenously.

Conclusions: Intravenous lidocaine started before and immediately after acid instillation attenuated the acute lung injury,

in part by inhibiting the sequestration and activation of neutrophils. (Key words: acid aspiration; acute respiratory distress syndrome; edema; immune response; local anesthetics; neutrophils; superoxide anion.)

ACID aspiration may produce severe, acute lung injury, the characteristics of which resemble those of acute respiratory distress syndrome.¹ The precise mechanism through which acid aspiration induces respiratory failure is not yet fully understood. However, cellular components (e.g., neutrophils, macrophages) and humoral mediators (e.g., cytokines) are thought to play a pivotal role in the pathogenesis of this acute lung injury.²⁻⁵ Neutrophils accumulated in the lung in response to chemotaxins adhere to pulmonary endothelial cells through the interaction of adhesion molecules, the expression of which is enhanced by cytokines.^{6,7} Neutrophils thereafter attack the endothelium by releasing protease, reactive oxygen species, and lipid metabolites.^{5,8,9}

Lidocaine has various inhibitory effects on neutrophil function, including chemotaxis¹⁰ and superoxide anion (O₂⁻) release.^{10,11} The drug has been shown to attenuate acute lung injury induced by endotoxin¹² or hyperoxia,¹³ probably by inhibiting the function of neutrophils. Lidocaine is also likely to inhibit eosinophil-active cytokines¹⁴ and the expression of cellular adhesion molecules on neutrophils.¹⁵ Thus lidocaine may be able to prevent or attenuate acute lung injury induced by acid aspiration.

To test this hypothesis, we assessed the effect of intravenous treatment with lidocaine on hydrochloric acid (HCl)-induced lung injury.

Materials and Methods

Animal Preparation and Protocol

This experiment was conducted according to the guidelines of the animal care review board of Kobe University School of Medicine.

Japanese White rabbits ($n = 56$; weight, 2.2-2.6 kg)

* Research Fellow in Anesthesiology.

† Assistant Professor in Anesthesiology.

‡ Instructor in Anesthesiology.

§ Associate Professor of Anesthesiology and Intensive Care Unit.

|| Professor and Chairman of Anesthesiology and Intensive Care Unit.

Received from the Departments of Anesthesiology and Intensive Care Unit, Kobe University School of Medicine, Kobe, Japan. Submitted for publication January 2, 1997. Accepted for publication January 14, 1998. Supported in part by departmental funds.

Address reprint requests to Dr. Mikawa: Department of Anesthesiology, Kobe University School of Medicine, Kusunoki-cho 7, Chuo-ku, Kobe, 650, Japan.

LIDOCAINE FOR HCL-INDUCED LUNG INJURY

were anesthetized with 15 mg/kg ketamine injected intravenously through a catheter inserted into an ear vein and were intubated with a 3.5-mm endotracheal tube through a tracheotomy. An arterial catheter was placed through a cutdown in the right femoral artery to monitor blood pressure and take samples for blood gas analysis and assay. The animals were paralyzed with pancuronium bromide, and anesthesia was maintained with a continuous infusion of ketamine ($10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$). The lungs of the animals were mechanically ventilated using an infant ventilator (model IV100B; Sechrist, Anaheim, CA) with an inspired oxygen concentration of 100%. The initial tidal volume was set to 10 ml/kg (measured by pneumotachograph) and positive end-expiratory pressure was 5 cm H_2O . The respiratory rate was controlled to produce an initial arterial carbon dioxide tension (Pa_{CO_2}) of 32–38 mmHg. Central venous pressure was also monitored using a catheter inserted into the femoral vein. The animals were placed on a heating pad under a radiant heat lamp so that the body temperature could be kept at 37.8–40.2°C at the esophagus. Lactated Ringer's solution was administered intravenously at a rate of $8 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$.

After 15 min of stabilization, baseline values of lung mechanics and hemodynamics were measured, and the arterial blood sample was taken to determine arterial oxygen tension (Pa_{O_2}), peripheral leukocyte counts, and plasma cytokine concentrations.

Study 1. Twenty-eight rabbits were randomly assigned to one of four groups ($n = 7$ each). The rabbits in the saline-lidocaine and HCl-lidocaine groups received bolus intravenous injections of 2 mg/kg lidocaine followed by continuous intravenous infusion of lidocaine at a rate of $2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$. Animals in the saline-saline and HCl-saline groups received the same volume of saline (injection and infusion). Ten minutes after pretreatment with lidocaine or saline, the rabbits in the HCl-saline and HCl-lidocaine groups received 3 ml/kg 0.1 N HCl intratracheally. The animals in the saline-saline and saline-lidocaine groups received intratracheal saline (3 ml/kg).

Study 2. Rabbits (4 groups, $n = 7$ each) received HCl (0.1 N, 3 ml/kg) intratracheally. Saline was administered 10 min before injury in the saline group. Administration of lidocaine was started 10 min before, 10 min after, or 30 min after acid aspiration in the lidocaine-pre, lidocaine-10 min, and lidocaine-30 min groups, respectively. The rationale for the dose of intravenous lidocaine treatment was based on results of past experiments.^{12,13,16,17}

Data on the hemodynamics and lung mechanics and blood sampling for arterial gas analysis and leukocyte counts were obtained every hour after HCl or saline intratracheal instillation. In all groups, arterial blood was drawn for the cytokine assay at 0, 2, 4, and 6 h, and the plasma was separated and stored at -70°C until assay. Plasma concentrations of interleukin-6 (IL-6) and IL-8 were measured by enzyme immunoassay (Amersham, Buckinghamshire, UK).

In groups receiving lidocaine, arterial blood samples were obtained 1, 2, 4, and 6 h after intratracheal instillation of HCl or saline. Plasma lidocaine concentrations were determined by fluorescence polarization immunoassay (TDX system, Abbott, North Chicago, IL).

Assessment of Acute Lung Injury

Analysis of Blood Samples and Lung Mechanics.

Arterial blood gases (Pa_{O_2} , Pa_{CO_2} , and pH) were analyzed using a blood gas analyzer (ABL2 Radiometer, Copenhagen, Denmark), and the number of peripheral leukocytes was measured using a Coulter counter (Sysmex K-1000; Toa Iyos Denchi, Kobe, Japan). Lung mechanics were measured by the passive expiratory flow-volume technique.¹⁸ Air flow was measured with a Flemish 00 pneumotachograph and a differential pressure transducer (model MP045; Validyne Engineering, Northridge, CA). Airway pressure was measured at the proximal end of the pneumotachometer with a semiconductor pressure transducer (model P-300 501G; Copal Electronics, Tokyo, Japan). The volume was determined for each breath by digital integration of airflow using a respiration monitor (Aivision, Tokyo, Japan). The compliance and resistance of the total respiratory system were calculated using a personal computer (PC9801 VM11; NEC, Tokyo, Japan).

At the end of the experiment (6 h after intratracheal HCl or saline infusion), the thorax was opened and blood (15 ml) was drawn into a heparinized syringe (20 U/ml) from the pulmonary artery for the chemiluminescence assay. Blood sampling was completed before the overdose injection of thiamylal by which the rabbits were killed. The heart and lungs were removed *en bloc* by observers blinded to the nature of the experiments.

Analysis of Bronchoalveolar Lavage Fluid.

Through the right mainstem bronchus of the removed lung, 35 ml saline with EDTA-2Na at 4°C was slowly infused and withdrawn. This procedure was repeated three times. Indomethacin was added to the bronchoalveolar lavage fluid (BALF) to inhibit the further metabolism of arachidonic acid to prostaglandins during analy-

sis. The BALF was analyzed for cell counts and differentiation. A cytocentrifuged preparation (Cytospin 2; Shandon Southern Products, Pittsburgh, PA) of the BALF was stained with Wright-Giemsa for cell differentiation. The cells present in the fluid were counted using the Sysmex K-1000 counter according to the Bürker-Türk method. The fluid was centrifuged at 250g at 4°C for 10 min to remove the cells. The cell-free supernatant was divided into several aliquots and stored at -70°C until the assay. The following substances, metabolites, and mediators in the BALF were measured: Albumin concentrations were determined by nephelometry with immunoglobulin G fraction of goat anti-rabbit albumin (Cappel, Durham, NC); concentrations of IL-6 and IL-8 were measured by enzyme immunoassay (Amersham); and concentration of thromboxane A₂ and prostacyclin were quantified by radioimmunoassay (Amersham) as thromboxane B₂ and 6-keto prostaglandin F1 α , the stable metabolites, respectively.

Wet-to-Dry Weight Ratio of the Lung. The left upper lobe of each rabbit was weighed and then dried to a constant weight at 60°C for 24 h in an oven. The ratio of wet weight to dry weight was calculated to assess tissue edema.

Chemiluminescence Assay

Reagents. Cypridina luciferin analog (2-methyl-6-phenyl-3,7-dihydroimidazo [1,2-a]-pyrazin-3-one), dimethyl sulfoxide, Hank's balanced salt solution (HBSS), Histopaque-1119, Histopaque-1077, N-formyl-L-methionyl-L-leucyl-L-phenylalanine (FMLP), and zymosan A were obtained from Sigma Chemical Company (St. Louis, MO). The cypridina luciferin analog was dissolved to 56 μ g/ml in distilled water. Five milligrams FMLP was dissolved in 1.14 ml dimethyl sulfoxide. Just before use, the stored solution was diluted with 50% DMSO-50% HBSS to 100 μ M. Zymosan A was opsonized according to the method of Nishida *et al.*¹⁹ with some modification. Briefly, zymosan A was suspended in HBSS at a concentration of 2 mg/ml and heated in a boiling water bath for 100 min, washed twice with HBSS, and opsonized with pooled serum in a shaking water bath for 30 min at 37°C. The opsonized zymosan was washed twice, resuspended in HBSS to a concentration of 20 mg/ml, and stored at -70°C until use.

Isolation of Neutrophils. Histopaque-1119, Histopaque-1077, and whole blood were layered and centrifuged at 700g for 30 min at room temperature. The layer containing granulocytes (at the interphase between Histopaque-1077 and Histopaque-1119) was

transferred to another tube. The cells were washed in HBSS and centrifuged twice at 200g for 10 min. The resultant leukocytes were suspended to 1×10^7 cells/ml in HBSS and kept at 0°C for no more than 3 h before use. The cell analysis showed that >97% of the cells were neutrophils, and the trypan blue dye exclusion test confirmed that >95% of the cells were viable.

Measurement of Chemiluminescence. Measurement of chemiluminescence was based on the method established by Sugioka *et al.*²⁰ The incubation mixture contained 4×10^5 leukocytes, 20 μ l FMLP, or 80 μ l opsonized zymosan, 25 μ l cypridina luciferin analog, and HBSS in a total volume of 2 ml. Cells and HBSS were preincubated for 3 min, and the reaction was initiated by the simultaneous addition of the other two components. Cypridina luciferin analog-dependent luminescence, which is thought to represent primarily superoxide anion production, was monitored using a luminescence reader (Lumicounter-1000; Nichion, Chiba, Japan). During luminescence measurement, the incubation mixture was agitated at 37°C in the luminescence reader. Ketamine used as an anesthetic in the current study is thought to have no effect on superoxide anion production by neutrophils at doses used in clinical settings.²¹

Histopathologic Examination

Immediately after the rabbits were killed, the left lower lobe was fixed by instillation of 10% formaldehyde solution through the left lower bronchus at 20 cm H₂O. The specimens were embedded in paraffin wax, stained with hematoxylin and eosin, and examined under a light microscope. Acute lung injury was scored by a blinded observer according to the following four items: (1) alveolar congestion, (2) hemorrhage, (3) infiltration or aggregation of neutrophils in airspace or the vessel wall, and (4) thickness of the alveolar wall/hyaline membrane formation. Each item was graded according to a five-point scale: 0 = minimal (little) damage, 1+ = mild damage, 2+ = moderate damage, 3+ = severe damage, and 4+ = maximal damage.¹²

Statistics

The lung injury score data are given as median (range), whereas the other data are expressed as mean \pm SD. Parametric data were analyzed using a one-way analysis of variance with the Tukey-Kramer test for between-group comparisons at each treatment interval and paired *t* tests for comparisons within groups. The lung

LIDOCAINE FOR HCL-INDUCED LUNG INJURY

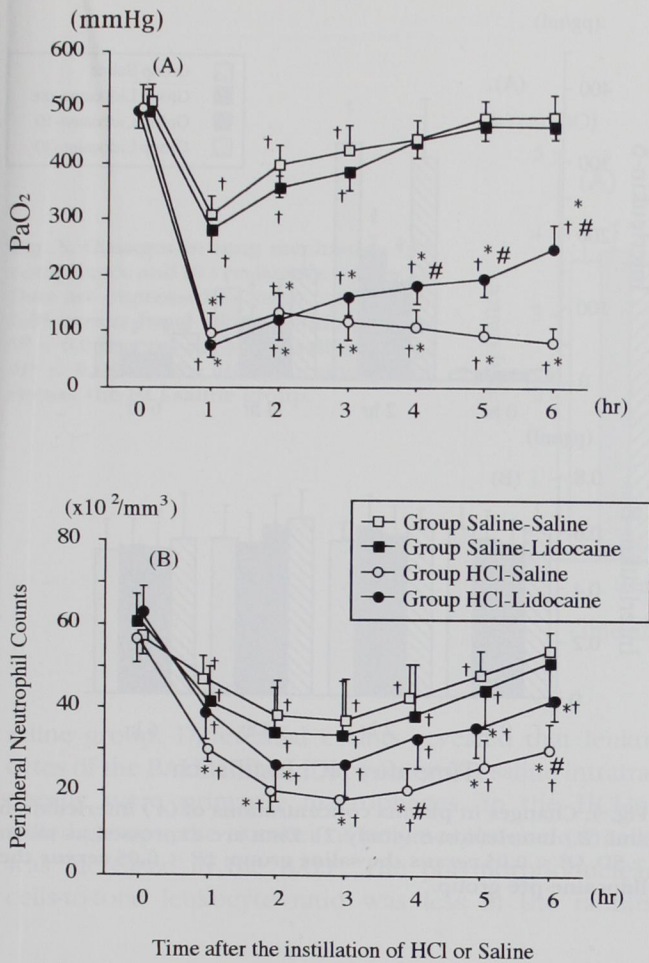


Fig. 1. Changes in (A) partial oxygen tension (P_{aO_2}) and (B) peripheral leukocyte count (study 1). Data are expressed as the mean \pm SD of seven rabbits per group. † $P < 0.05$ versus basal values within groups; * $P < 0.05$ versus the saline-saline group; # $P < 0.05$ for the HCl-lidocaine group versus the HCl-saline group.

injury score was analyzed using the Kruskal-Wallis rank test. Probability values < 0.05 were deemed significant.

Results

No rabbits died during the 6-h study period. In groups receiving lidocaine, plasma lidocaine concentrations were maintained between 1.2–2.5 $\mu\text{g/ml}$.

Changes in Oxygen Tension, Hemodynamics, and Peripheral Leukocyte Counts

As shown in figures 1 and 2, intratracheal instillation of HCl decreased P_{aO_2} dramatically within 1 h. Thereaf-

ter, P_{aO_2} in the HCl-saline group remained at a low level with gradual reduction until 6 h after HCl instillation. Lidocaine treatment failed to prevent the initial decrease in P_{aO_2} . Lidocaine before and 10 min after instillation of HCl promoted partial recovery from deteriorated oxygenation, although the drugs given 30 min after insult were not effective. In contrast, P_{aO_2} decreased transiently in rabbits receiving saline instillation, but it readily returned toward the basal value (fig. 1).

Although arterial pressure and heart rate increased in response to HCl or saline instillation, there was no difference among the groups at any point (data not shown). The central venous pressure did not differ among the groups.

Peripheral leukocyte counts gradually decreased with

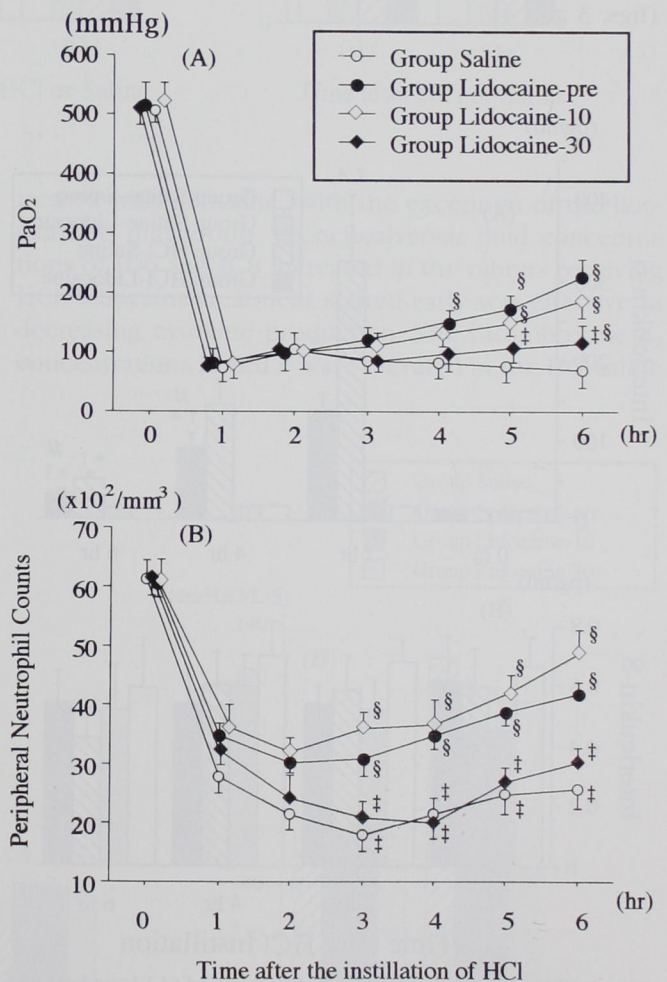


Fig. 2. Changes in (A) the partial oxygen tension P_{aO_2} and (B) peripheral leukocyte count (study 2). Data are expressed as the mean \pm SD of seven rabbits per group. § $P < 0.05$ versus the saline group; ‡ $P < 0.05$ versus the lidocaine-pre group.

instillation of saline or HCl and reached the lowest level 3 h after administration (fig. 1B). The leukopenia was more severe in rabbits receiving HCl than in those that received saline. Lidocaine treatment before and 10 min after HCl aspiration slightly, but significantly, attenuated the peripheral leukopenia (fig. 2B).

Changes in Plasma Cytokine Levels

Plasma concentrations of IL-6 increased after intratracheal HCl instillation, with a peak occurring at 2 h, although plasma IL-8 concentrations remained unchanged in all of the groups. The plasma concentration of IL-6 was significantly decreased by intravenous lidocaine treatment before and 10 min after HCl administration. As with Pa_{O_2} changes, lidocaine given 30 min after HCl instillation was not effective against IL-6 elevation (figs. 3 and 4).

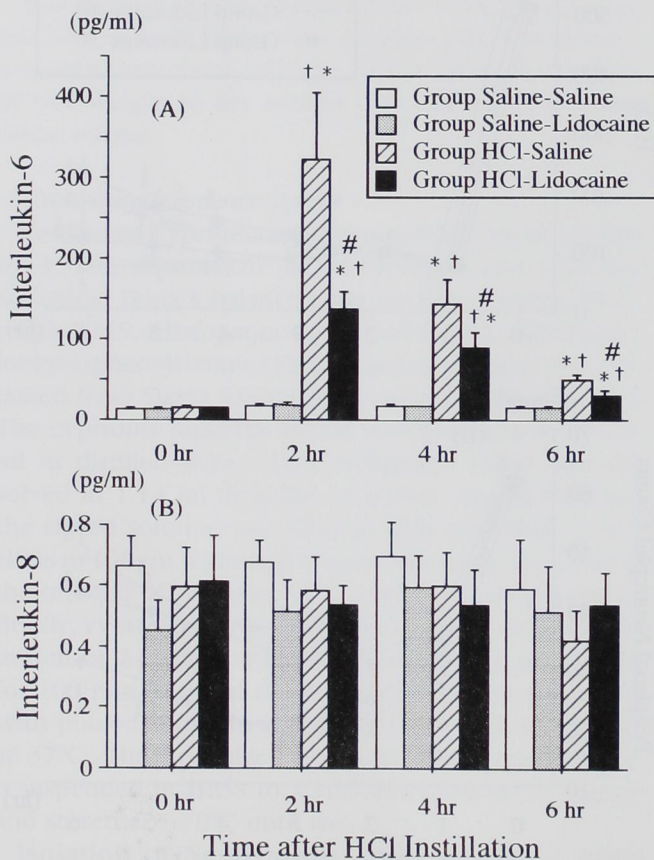


Fig. 3. Changes in plasma concentrations of (A) interleukin-6 and (B) interleukin-8 (study 1). Data are expressed as mean \pm SD. † P < 0.05 versus basal values within groups; * P < 0.05 versus the saline-saline group; # P < 0.05 for the HCl-lidocaine group versus the HCl-saline group.

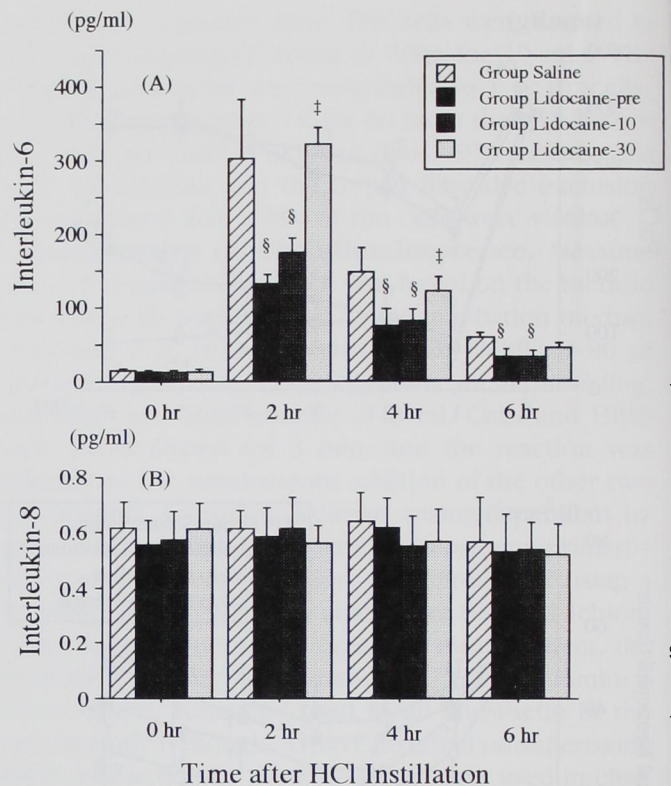


Fig. 4. Changes in plasma concentrations of (A) interleukin-6 and (B) interleukin-8 (study 2). Data are expressed as mean \pm SD. § P < 0.05 versus the saline group; † P < 0.05 versus the lidocaine-pre group.

Lung Mechanics

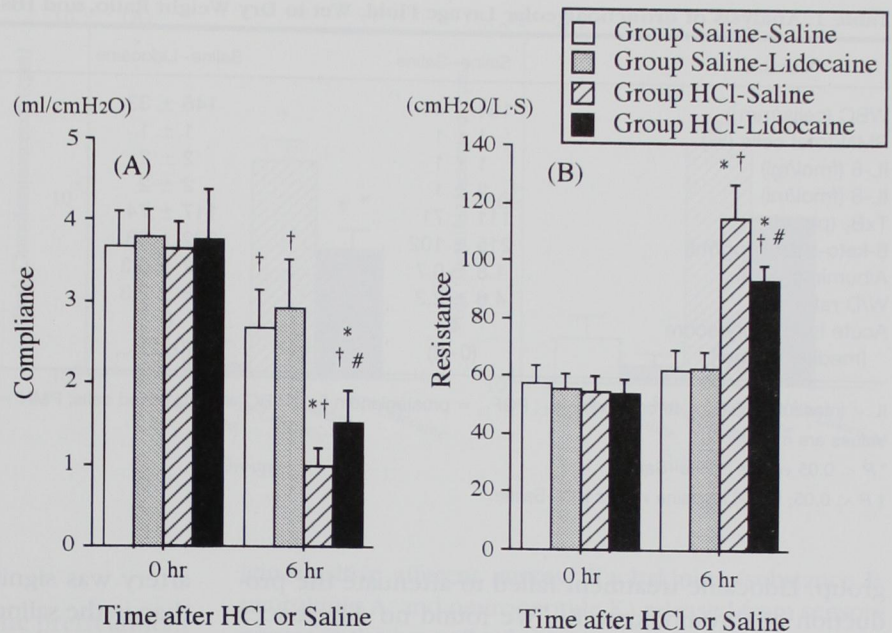
Neither compliance nor resistance immediately after the start of mechanical ventilation was different among the four groups. Compliance immediately before the end of the study was lower in the HCl-treated rabbits than in the saline-instilled groups. Intratracheal instillation of HCl increased the resistance. Lidocaine prevented the reduction of compliance and the increase in resistance induced by HCl, although lidocaine given 30 min after insult was not significantly effective (figs. 5 and 6).

Analysis of Bronchoalveolar Lung Fluid and Lung Edema

Recovered volume of BALF in the groups was 84–90 ml (80–86% of infused saline) and was not different among the groups. The total number of leukocytes recovered in BALF was greater in the groups receiving HCl than in the intratracheal saline groups (table 1). As shown in table 2, the leukocyte counts were less in the lidocaine-pre and lidocaine-10 min groups than in the

LIDOCAINE FOR HCL-INDUCED LUNG INJURY

Fig. 5. Changes in lung mechanics: (A) compliance and (B) resistance (study 1). Data are expressed as mean \pm SD. † $P < 0.05$ versus basal values within groups; * $P < 0.05$ versus the saline-saline group; # $P < 0.05$ for the HCl-lidocaine group versus the HCl-saline group.



saline group. Differential counts revealed that leukocytes of the BALF in the groups receiving saline intratracheally were primarily macrophages. In the HCl-instilled groups, the number of polymorphonuclear cells was increased in the BALF. The polymorphonuclear cells-to-total leukocyte ratio was less in the rabbits

treated with lidocaine, with the exception of the lidocaine-30 min group. Bronchoalveolar fluid concentrations of IL-6 and IL-8 increased in the rabbits receiving HCl. Lidocaine treatment started early was effective in decreasing cytokine production. The thromboxane B₂ concentrations in BALF were elevated in the HCl-saline

Fig. 6. Changes in lung mechanics: (A) compliance and (B) resistance (study 2). Data are expressed as mean \pm SD. § $P < 0.05$ versus the saline group; ‡ $P < 0.05$ versus the lidocaine-pre group.

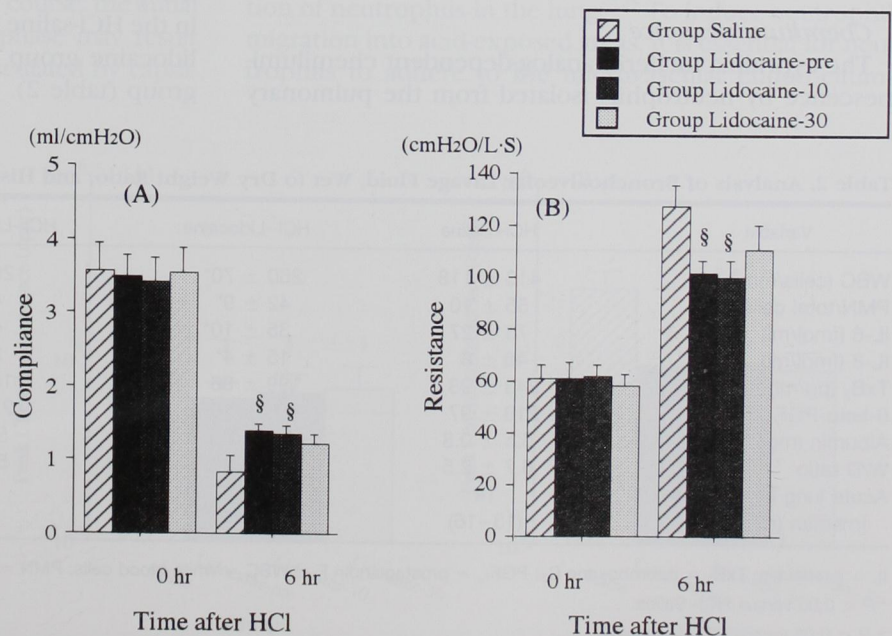


Table 1. Analysis of Bronchoalveolar Lavage Fluid, Wet to Dry Weight Ratio, and Histological Examination (Study 1)

Variable	Saline-Saline	Saline-Lidocaine	HCl-Saline	HCl-Lidocaine
WBC (cells/mm ³)	154 ± 44	146 ± 33	403 ± 101*	255 ± 73*†
PMN/total cells (%)	1 ± 1	1 ± 1	57 ± 9*	41 ± 10*
IL-6 (fmol/ml)	1 ± 1	2 ± 2	71 ± 25*	38 ± 10*†
IL-8 (fmol/ml)	2 ± 1	2 ± 2	43 ± 8*	14 ± 7*†
TxB ₂ (pg/ml)	111 ± 71	117 ± 74	232 ± 95*	186 ± 87*
6-keto-PGF _{1α} (pg/ml)	215 ± 102	232 ± 88	235 ± 94	221 ± 112
Albumin (mg/dl)	1.8 ± 0.7	1.2 ± 0.3	6.4 ± 0.7*	5.1 ± 0.7*†
W/D ratio	4.6 ± 2.2	4.8 ± 2.3	6.5 ± 2.7*	5.5 ± 2.6*†
Acute lung injury score [median (range)]	0 (0-2)	0 (0-2)	14* (13-16)	10*† (7-14)

IL = interleukin; TxB₂ = thromboxane B₂; PGF_{1α} = prostaglandin F_{1α}; WBC = white blood cells; PMN = polymorphonuclear neutrophils.

Values are mean ± SD.

* *P* < 0.05 versus Saline-Saline.

† *P* < 0.05, HCl-Lidocaine versus HCl-Saline.

group. Lidocaine treatment failed to attenuate the production of thromboxane B₂. We found no differences in 6-keto-prostaglandin F_{1α} levels in the BALF among the groups. The lung wet-to-dry weight ratio was calculated as a measurement of lung edema. The ratio increased in the rabbits receiving HCl. Albumin concentrations in the supernatant of BALF were higher in the HCl-instilled rabbits than in saline-instilled rabbits. Lidocaine pretreatment attenuated the increase in the wet-to-dry weight ratio and albumin concentrations. Early post-treatment with lidocaine was as effective as pre-treatment, but lidocaine given 30 min later was not.

Chemiluminescence

The cypridina luciferin analog-dependent chemiluminescence by neutrophils isolated from the pulmonary

artery was significantly higher in the HCl-saline group than in the saline-saline group when stimulated by opsonized zymosan or FMLP (fig. 7). Chemiluminescence was attenuated in all of the lidocaine-treated groups.

Histopathologic Analysis

Light microscopic findings in the HCl-saline group included edema, ruptured and thickened alveolar walls, and the presence of inflammatory cells and erythrocytes in alveolar spaces. Alveoli also contained diffuse proteinaceous exudate. In contrast, these changes were less pronounced in the HCl-lidocaine group. The score in the HCl-saline group was greater than that in the HCl-lidocaine group (table 1) and in the lidocaine-10 min group (table 2).

Table 2. Analysis of Bronchoalveolar Lavage Fluid, Wet to Dry Weight Ratio, and Histological Examination (Study 2)

Variable	HCl-Saline	HCl-Lidocaine	HCl-Lidocaine 10 min	HCl-Lidocaine 30 min
WBC (cells/mm ³)	413 ± 118	260 ± 70*	298 ± 92	334 ± 97
PMN/total cells (%)	55 ± 10	42 ± 9*	40 ± 10*	48 ± 9
IL-6 (fmol/ml)	73 ± 27	35 ± 10*	44 ± 18*	62 ± 22†
IL-8 (fmol/ml)	40 ± 8	15 ± 4*	21 ± 5*	37 ± 4†
TxB ₂ (pg/ml)	243 ± 93	199 ± 86	186 ± 80	194 ± 85
6-keto-PGF _{1α} (pg/ml)	219 ± 97	205 ± 109	218 ± 81	239 ± 107
Albumin (mg/dl)	6.5 ± 0.8	4.9 ± 0.7*	5.0 ± 0.7*	6.2 ± 0.9†
W/D ratio	6.7 ± 2.5	5.4 ± 2.5*	5.6 ± 2.4*	6.3 ± 2.9†
Acute lung injury score [median (range)]	14 (13-16)	10* (7-13)	11* (8-14)	14† (11-16)

IL = interleukin; TxB₂ = thromboxane B₂; PGF_{1α} = prostaglandin F_{1α}; WBC = white blood cells; PMN = polymorphonuclear neutrophils.

* *P* < 0.05 versus HCl-Saline.

† *P* < 0.05 versus HCl-Lidocaine.

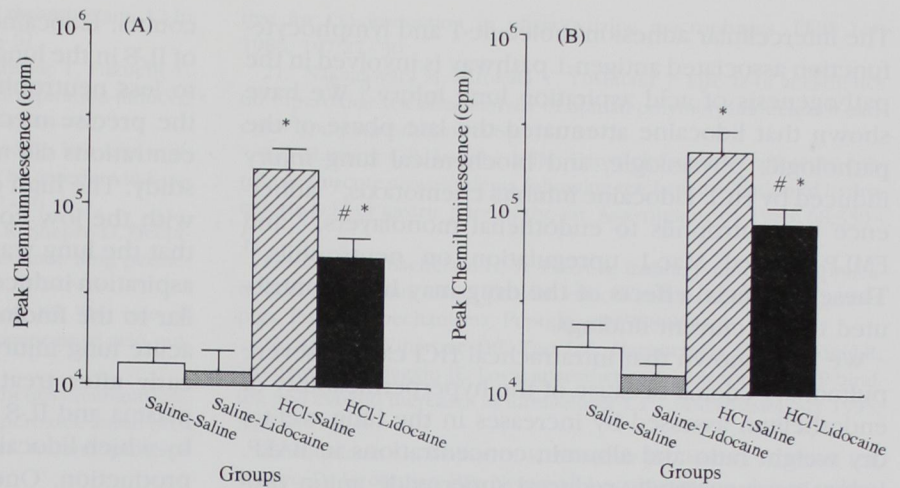


Fig. 7. Cypridina luciferin analog-dependent chemiluminescence (peak) by neutrophils isolated from the pulmonary artery (study 1). Data are expressed as mean \pm SD. (A) Opsonized zymosan-stimulated chemiluminescence, (B) N-formyl-L-methionyl-L-leucyl-L-phenylalanine-stimulated chemiluminescence. * $P < 0.05$ versus the saline-saline group; # $P < 0.05$ for the HCl-lidocaine group versus the HCl-saline group.

Discussion

The current study showed that lidocaine pretreatment promoted the recovery of PaO₂ after HCl instillation, although the drug did not prevent the initial decrease in PaO₂. This alleviating effect on lung injury by lidocaine was accompanied by improved lung mechanics and the decrease of cytokines in BALF, the albumin concentration in BALF, and sequestration of neutrophils into the lung. The efficacy of post-treatment with lidocaine depended on the timing of administration: 10 min after the insult was effective, but 30 min after was not.

Hydrochloric acid instillation caused lung injury by two different pathways according to the time course: the initial phase and the late phase.² The initial phase may result from a physiochemical process or be mediated by capsa-

icin-sensitive afferent nerves.² Tachykinins (substance P, neurokinin A, and neuropeptide K) released from sensory nerves in the lung after irritation with capsaicin are active in inducing airway mucosal edema.²² Although lidocaine inhibits capsaicin-sensitive nerve activity²³ and tachykinin-mediated neurotransmission,²⁴ the drug failed to attenuate the initial phase of lung injury characterized by the dramatic decrease in PaO₂.

The late phase of the injury, which pathologically mimics acute respiratory distress syndrome, is mediated by neutrophils and is consistent with an acute inflammatory response.² In this pathway, chemotaxins, including IL-8 and thromboxane A₂, promote the sequestration of neutrophils in the lung.^{7,25} To induce neutrophil migration into acid-exposed areas, it is essential for neutrophils to adhere to the microvascular endothelium.

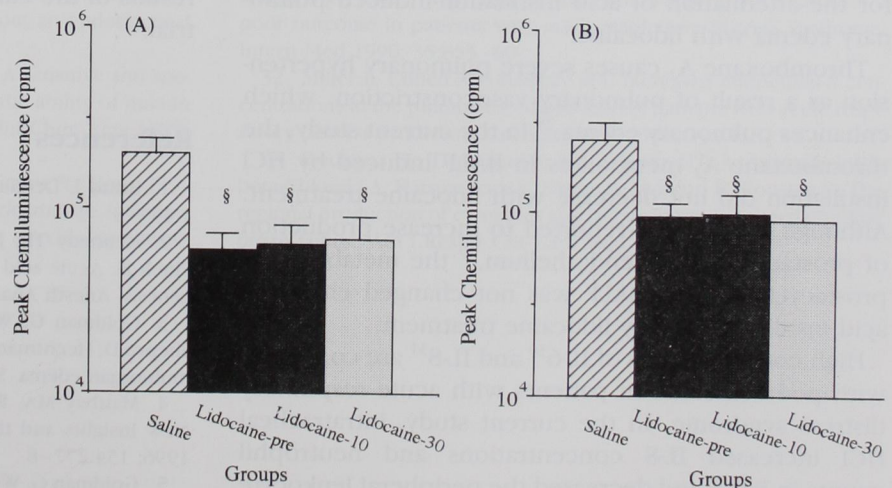


Fig. 8. Cypridina luciferin analog-dependent chemiluminescence (peak) by neutrophils isolated from the pulmonary artery (study 2). Data are expressed as mean \pm SD. (A) Opsonized zymosan-stimulated chemiluminescence, (B) N-formyl-L-methionyl-L-leucyl-L-phenylalanine-stimulated chemiluminescence. § $P < 0.05$ versus the saline group.

The intercellular adhesion molecule-1 and lymphocyte-function associated antigen-1 pathway is involved in the pathogenesis of acid aspiration lung injury.⁶ We have shown that lidocaine attenuated the late phase of the pathologic, physiologic, and biochemical lung injury induced by HCl. Lidocaine inhibits chemotaxis,¹⁰ adherence of neutrophils to endothelial monolayers,¹⁶ and FMLP-induced Mac-1 upregulation on neutrophils.¹⁵ These beneficial effects of the drug may have contributed to our current findings.

We have shown that intratracheal HCl caused severe pulmonary edema because of the hyperpermeability of endothelium assessed by increases in the lung wet-to-dry weight ratio and albumin concentrations in BALF. Lidocaine successfully reduced superoxide anion production by neutrophils isolated from rabbits receiving HCl. We believe that lidocaine lessened endothelial damage, in part by reducing superoxide anion release from neutrophils, and consequently attenuated pulmonary edema. This ability of lidocaine was observed in our previous studies using other experimental acute respiratory distress syndrome models.^{12,13} Further, superoxide anion indirectly contributes to edematous lung injury by inactivating antiproteases.²⁶ Although superoxide anion production at 6 h was suppressed in the HCl-lidocaine-30 min group, hyperpermeability was not attenuated. The early activation of neutrophils may be critical for the attenuation of endothelial damage leading to pulmonary edema. Lidocaine has been shown to decrease the increases in pulmonary vascular protein leak induced by thiourea through a mechanism that is independent of the effects on neutrophil oxygen metabolite-dependent toxicity, although the details are not understood.²⁷ This mechanism may also be responsible for the attenuation of acid instillation-induced pulmonary edema with lidocaine.

Thromboxane A₂ causes severe pulmonary hypertension as a result of pulmonary vasoconstriction, which enhances pulmonary edema.²⁸ In the current study, the thromboxane A₂ metabolites in BALF induced by HCl instillation did not decrease with lidocaine treatment. Although lidocaine is reported to increase production of prostacyclin from endothelium,²⁹ the metabolite of prostacyclin in the BALF was not changed either by acid instillation nor by lidocaine treatment.

High concentrations of IL-6³⁰ and IL-8³¹ are correlated with poor outcome in patients with acute respiratory distress syndrome. In the current study, intratracheal HCl increased IL-8 concentrations and neutrophil counts in BALF and decreased the peripheral leukocyte

counts. Lidocaine successfully decreased the secretion of IL-8 in the lung after HCl instillation, probably leading to less neutrophil sequestration in the lung, although the precise mechanism is unknown. Plasma IL-8 concentrations did not increase after HCl instillation in our study. The high concentrations of IL-8 in BALF coupled with the low concentrations of IL-8 in plasma suggest that the lung was the primary source of IL-8 in the acid aspiration-induced lung injury. This phenomenon is similar to the findings in a clinical study of sepsis-induced acute lung injury.³² In our study, lidocaine before and early after treatment suppressed elevation of IL-6 in plasma and IL-8 in BALF. We propose two hypotheses by which lidocaine successfully attenuated the cytokine production. One is that lidocaine directly suppressed cytokine production from many kinds of cells, including macrophages, alveolar epithelium, and endothelium. There has been no report confirming that lidocaine attenuates production of proinflammatory cytokines. The second hypothesis is that lidocaine attenuated the inflammatory response, resulting in less production of cytokines. IL-6 is secreted from the most severely affected organs, as the higher concentration of IL-6 was measured in the drainage vein of the injured organ (lung or liver) than in the peripheral venous blood.³³ Lidocaine might decrease cytokine production by attenuating the inflammatory response.

In conclusion, pre- or early post-treatment with lidocaine attenuated the late phase of acid instillation-induced lung injury in rabbits, probably by inhibiting the sequestration and activation of neutrophils. Lidocaine, which has been used extensively in clinical practice, is not costly, has a good safety record, and is easily obtained. These advantages lead us to conclude that the results of the current study may be a basis for a clinical trial.

References

1. Britto J, Demling RH: Aspiration lung injury. *New Horiz* 1996; 1:435-9
2. Kennedy TP, Johnson KJ, Kunkel RG, Ward PA, Knight PR, Finch JS: Acute acid aspiration lung injury in the rat: Biphasic pathogenesis. *Anesth Analg* 1989; 69:87-92
3. Goldman G, Welbourn R, Klausner JM, Kobzik L, Valeri CR, Shepro D, Hechtman HB: Leukocytes mediate acid aspiration-induced multiorgan edema. *Surgery* 1993; 114:13-20
4. Matthay MA, Rosen GD: Acid aspiration induced lung injury. New insights and therapeutic options. *Am J Respir Crit Care Med* 1996; 154:277-8
5. Goldman G, Welbourn R, Kobzik L, Valeri CR, Shepro D, Hecht-

LIDOCAINE FOR HCL-INDUCED LUNG INJURY

- man HB: Synergism between leukotriene B₄ and thromboxane A₂ in mediating acid-aspiration. *Surgery* 1992; 111:55-61
6. Nagase T, Ohga E, Sudo E, Katayama H, Matsuse T, Fukuchi Y: Intercellular adhesion molecule-1 mediates acid aspiration-induced lung injury. *Am J Respir Crit Care Med* 1996; 154:504-10
 7. Folkesson HG, Matthay MA, Hebert CA, Broaddus VC: Acid aspiration-induced lung injury in rabbit is mediated by interleukin-8-dependent mechanisms. *J Clin Invest* 1995; 96:107-16
 8. Goldman G, Welbourn R, Kobzik L, Valeri CR, Shepro D, Hechtman HB: Reactive oxygen species and elastase mediate lung permeability after acid aspiration. *J Appl Physiol* 1992; 73:571-5
 9. Knight PR, Druskovich G, Tait AR, Johnson KJ: The role of neutrophils, oxidants, and proteases in the pathogenesis of acid pulmonary injury. *ANESTHESIOLOGY* 1992; 77:772-8
 10. Sasagawa S: Inhibitory effects of local anesthetics on migration, extracellular release of lysosomal enzyme, and superoxide anion production in human polymorphonuclear leukocytes. *Immunopharmacol Immunotoxicol* 1991; 13:607-22
 11. Peck SL, Johnston RB Jr, Horwitz LD: Reduced neutrophil superoxide anion release after prolonged infusions of lidocaine. *J Pharmacol Exp Ther* 1985; 235:418-22
 12. Mikawa K, Nishina K, Maekawa N, Takao Y, Yaku H, Obara H: Effect of lidocaine pretreatment on endotoxin-induced lung injury in rabbits. *ANESTHESIOLOGY* 1994; 81:689-99
 13. Takao Y, Mikawa K, Nishina K, Maekawa N, Obara H: Lidocaine attenuates hyperoxic lung injury in rabbits. *Acta Anaesthesiol Scand* 1996; 40:318-25
 14. Ohnishi T, Kita H, Maeno AN, Okada S, Sur S, Broide DH, Gleich GJ: Lidocaine in bronchoalveolar lavage fluid (BALF) is an inhibitor of eosinophil-active cytokines. *Clin Exp Immunol* 1996; 104:325-31
 15. Ohsaka A, Saionji K, Sato N, Igarashi J: Local anesthetic lidocaine inhibits the effect of granulocyte colony-stimulating factor on human neutrophil functions. *Exp Hematol* 1994; 22:460-6
 16. MacGregor RR, Thorner RE, Wright DM: Lidocaine inhibits granulocyte adherence and prevents granulocyte delivery to inflammatory sites. *Blood* 1980; 56:203-9
 17. Siminiak T, Wysocki H, Veit A, Maurer HR: The effect of selected antiarrhythmic drugs on neutrophil free oxygen radicals production measured by chemiluminescence. *Basic Res Cardiol* 1991; 86:355-62
 18. LeSouef PN, England SJ, Bryan AC: Total resistance of the respiratory system in preterm infants with and without an endotracheal tube. *J Pediatr* 1984; 104:108-11
 19. Nishida A, Kimura H, Nakano M, Goto T: A sensitive and specific chemiluminescence method for estimating the ability of human granulocytes and monocytes to generate O₂⁻. *Clin Chim Acta* 1989; 179:177-82
 20. Sugioka K, Nakano M, Kurashige S, Akuzawa Y, Goto T: A chemiluminescent probe with a Cypridina luciferin analog, 2-methyl-6-phenyl-3,7-dihydroimidazo-[1,2-a]pyrazin-3-one, specific and sensitive for O₂-production in phagocytizing macrophages. *FEBS Lett* 1986; 197:27-30
 21. Nakagawara M, Hirokata Y, Yoshitake J: Effects of anesthetics on superoxide releasing activity of human polymorphonuclear leukocytes. *Jpn J Anesthesiol* 1985; 34:754-9
 22. Martling C-R, Lundberg JM: Capsaicin sensitive afferents contribute to acute airway edema following tracheal instillation of hydrochloric acid or gastric juice in the rat. *ANESTHESIOLOGY* 1988; 68:350-6
 23. Rioux F, Bachelard H, St. Pierre S, Barabe J: Epicardial application of bradykinin elicits pressor effects and tachycardia in guinea pigs. Possible mechanisms. *Peptides* 1987; 8:863-8
 24. Li YM, Wingrove DE, Too HP, Marnnerakis M, Stimson ER, Strichartz GR, Maggio JE: Local anaesthetics inhibit substance P binding and evoked increases in intracellular Ca²⁺. *ANESTHESIOLOGY* 1995; 82:166-73
 25. Goldman G, Welbourn R, Klausner JM, Kobzik L, Valeri CR, Shepro D, Hechtman HB: Neutrophil accumulations due to pulmonary thromboxane synthesis mediate acid aspiration injury. *J Appl Physiol* 1991; 70:1511-7
 26. Lamy M, Deby-Dupont G, Deby C, Pincemail J, Duchateau J, Braun M, Damas P, Roth M: Measurements of mediator cascade during adult respiratory distress syndrome, Adult Respiratory Distress Syndrome. Edited by A Artigas, F Lemaire, PM Suter, WM Zapol. New York, Churchill Livingstone, 1992, pp 71-88
 27. Stelzner TJ, Welsh CH, Berger E, McCullough RG, Morris K, Repine JE, Weil JV: Antiarrhythmic agents diminish thiourea-induced pulmonary vascular protein leak in rats. *J Appl Physiol* 1987; 63:1877-83
 28. Heffner JE, Shoemaker SA, Canham EM, Patel M, McMurtry IF, Morris HG, Repine JE: Acetyl glyceryl ether phosphorylcholine-stimulated human platelets cause pulmonary hypertension and edema in isolated rabbit lungs. Role of thromboxane A₂. *J Clin Invest* 1983; 71:351-7
 29. Jones G, Hurley JV: The effect of prostacyclin on the adhesion of leukocytes to injured vascular endothelium. *J Pathol* 1984; 142:51-9
 30. Headley AS, Tolley E, Meduri GU: Infections and the inflammatory response in acute respiratory distress syndrome. *Chest* 1997; 111:1306-21
 31. Ikuta N, Taniguchi H, Kondoh Y, Takagi K, Hayakawa T: Sustained high levels of circulatory interleukin-8 are associated with a poor outcome in patients with adult respiratory distress syndrome. *Intern Med* 1996; 35:855-60
 32. Miller E, Cohen AB, Matthay MA: Increased interleukin-8 concentrations in the pulmonary edema fluid of patients with acute respiratory distress syndrome. *Crit Care Med* 1996; 24:1448-54
 33. Douzinas EE, Tsidemiadou PD, Pitaridis MT, Andrianakis I, Bobota-Chloraki A, Katsouyanni K, Sfyras D, Malagari K, Roussos C: The regional production of cytokines and lactate in sepsis-related multiple organ failure. *Am J Respir Crit Care Med* 1997; 155:53-9